

HATCHING AND LARVAL DEVELOPMENT OF *HAPLOSTOMELLA*  
*AUSTRALIENSIS* GOTTO (COPEPODA, FAM. ASCIDICOLIDAE),  
A PARASITE OF THE ASCIDIAN *STYELA ETHERIDGII* HERDMAN

D. T. ANDERSON and G. T. ROSSITER

*School of Biological Sciences, University of Sydney*

[Read 27th November, 1968]

*Synopsis*

*H. australiensis* produces paired cylindrical ovisacs each containing 70–100 eggs. Hatching occurs as a lecithotrophic, positively phototropic nauplius which swims briefly then moults to a demersal first copepodite. This stage exhibits sexual dimorphism and is probably the infective stage of the life cycle. Hatching is osmotic in the usual copepod manner and is stimulated by light and mechanical agitation. The obligatory light stimulus ensures that hatching occurs after the ovisac is discharged from the host. The response to mechanical agitation ensures a synchronous hatching which ruptures the tough wall of the ovisac. The hatching mechanism is specialized and the larval development is abbreviated compared with those of other ascidicolous copepods.

INTRODUCTION

During the course of a general survey of copepods of the Sydney area, in search of species suitable for embryological study, the authors collected specimens of a large worm-like parasitic copepod inhabiting the oesophagus and stomach of the simple ascidian *Styela etheridgii* Herdman. The parasite was identified by Dr. R. V. Gotto of Queen's University, Belfast, as a new species of the genus *Haplostomella* Chatton and Harant 1924, family Ascidicolidae. The four previously described species of *Haplostomella* have all been collected from northern hemisphere localities, where they occur in compound ascidians. The new species, named *H. australiensis* Gotto, has been described by Gotto (1968). In the present paper, we present observations on the general breeding activity, hatching and larval development of this animal.

MATERIALS AND METHODS

Adult females of *H. australiensis* were collected at intervals during June, July and August, 1967. The animals were taken from specimens of *Styela etheridgii* living at low tide level on Bottle and Glass Rocks on the south shore of Sydney Harbour. Records were kept of the frequency of occurrence of egg masses attached to the animals, and the later embryonic development, hatching and first two larval stages were studied using egg masses cultured in the laboratory.

The egg masses were cultured in glass tubes, the lower ends of which were closed with fine nylon gauze and immersed in well-aerated, filtered sea water. The water was changed regularly and tubes cleaned of algal film every 2 days. The cultures were maintained at 20°C. The water temperature in the natural habitat of the copepods at the time of collection ranged from 13 to 18°C.

The aquarium containing the culture tubes was at no time subjected to direct sunlight, but the laboratory was illuminated through windows on its northerly aspect during the day. Fluorescent lighting supplemented natural

illumination during working hours. At all times, the culture tubes were shaded from light from above by a sheet of white paper placed over their upper ends. Mention is made of these facts because it was subsequently determined that the embryos are sensitive to bright light stimulation as a causative factor in hatching.

The sequence of events in hatching was observed for individual embryos and for whole egg masses. The finer details of hatching of individual embryos, including the rupture of egg membranes and accurate measurements of changes in volume, were recorded by mounting eggs in sea water on a cavity slide sealed with a cover slip and observing hatching by phase contrast microscopy.

The sensitivity of the embryos to bright light stimulation was studied using a sharply focussed microscope lamp to deliver either flashes or longer periods of bright light stimulation. By this technique, the days on which embryos could be induced to hatch by stimulation with bright light were determined, relative to the day of hatching of unstimulated embryos of the same age. This difference is referred to in the following account as days before "normal" hatching.

The hatching response of embryos in solutions of different osmotic strengths was also studied, in accordance with the view expressed by Marshall and Orr (1954) and Davis (1959) that a common osmotic mechanism underlies hatching in copepods. Embryos were immersed in hypotonic or hypertonic sea water in Petri dishes brightly illuminated under a binocular

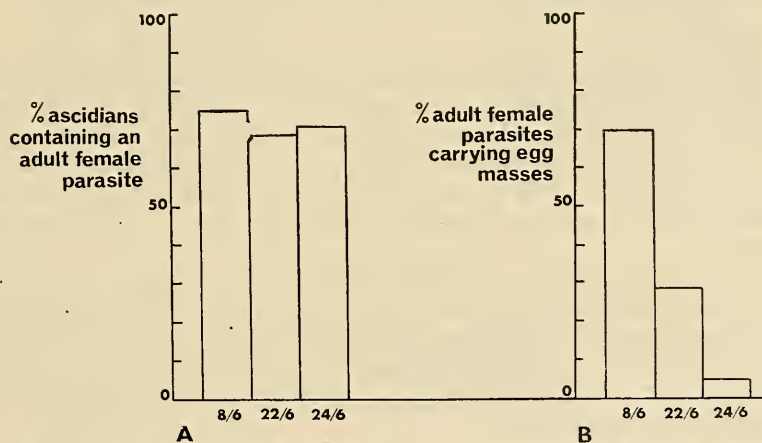


Fig. 1 A, % occurrence of adult female *Haplostomella* in three samples of *Styela* from a single population; B, % of parasites in each sample bearing egg masses.

microscope. Newly emerged nauplii, or the embryos if hatching failed to occur within a fixed period, were then transferred back to normal sea water. Controls remained in normal sea water throughout. Hypotonic sea water, osmotically equivalent to a 2.4% salt solution, was prepared by diluting sea water (3.5% salt solution) to 70% with distilled water. Hypertonic sea water (7.5% salt solution) was prepared by adding 4 gm. sodium chloride per 100 ml. to normal sea water.

## RESULTS

### *Occurrence and breeding of adult females*

The percentage occurrence of *H. australiensis* females in the ascidian *Styela etheridgii* in three separate samples collected at Bottle and Glass

Rocks is shown in Fig. 1A. The percentage, in each sample, of females which carried egg masses is shown in Fig. 1B. The decline in the percentage of ovigerous females in the second and third samples suggests a trend towards termination of a breeding cycle. The number of ascidians examined in the samples were 16, 75 and 88 respectively.

In none of the ascidians examined was there more than one adult female *Haplostomella*. The minimum length of the specimens was 4 mm., while the average length was 5 mm.

#### *Culturing of egg masses and larvae*

Most of the difficulties previously associated with the laboratory culture of egg masses from parasitic copepods (Wilson 1905, 1907a, 1907b, 1911a, 1911b) were experienced again in the present work. From the culture of 32 egg masses, only 6 pairs of masses already containing embryos at an advanced stage of development when collected remained viable until hatching. From 3 of these masses, embryos which had received no form of artificial stimulation hatched after 9, 13 and 17 days of culture respectively. Egg masses with embryos at very early stages of development, for example, cleavage or gastrulation, remained viable in culture for about 15 days. Maintenance of the hatched larval stages in the laboratory also proved difficult. About 95% of the nauplii hatched from egg masses in the laboratory failed to survive the first moult into a first copepodite stage. Because of the low survival rate, the few first copepodites which did emerge were fixed while intact, and development was not followed beyond this stage.

#### *The egg masses*

*H. australiensis*, like many copepods, lays its eggs into paired cylindrical ovisacs attached to the genital apertures of the female. The eggs when laid are spherical and  $180\mu$  in diameter. Seventy to 100 eggs are closely packed within each ovisac, which measures about 3 mm.  $\times$  0.8 mm. The eggs tend to be flattened slightly in the early stages of development, due to their close packing. They then gradually assume a regular ellipsoidal shape,  $200\mu$  long and  $160\mu$  in diameter.

The ovisacs function as brood chambers and remain attached to the female at least until the embryos reach a late stage of development. The period of development is estimated to be 6–8 weeks, taking into account the observation of 2 weeks of early development and  $2\frac{1}{2}$  weeks of later development of embryos in culture. No ovisacs with embryos at an intermediate stage of development were collected.

#### *The newly hatched larva*

The newly hatched nauplius of *H. australiensis*,  $200\mu$  in length, is illustrated in Fig. 2G. The nauplius is free-swimming and lecithotrophic. The antennules, antennae and mandibles all bear swimming setae, but feeding spines are absent. The labrum is also poorly defined. The post-naupliar region is large and swollen, but shows no external signs of segmentation or limb rudiments. The nauplius is markedly phototropic and is a very rapid swimmer.

#### *The sequence of events in hatching*

Towards the end of embryonic development, for almost a week before hatching, the embryo shows peristaltic movements of the gut and irregular, rather infrequent twitching of the body. Just before hatching is initiated,



there is a marked increase in activity. For a period of one or two minutes, the embryo exhibits vigorous movements of the whole body and especially of the three pairs of limbs.

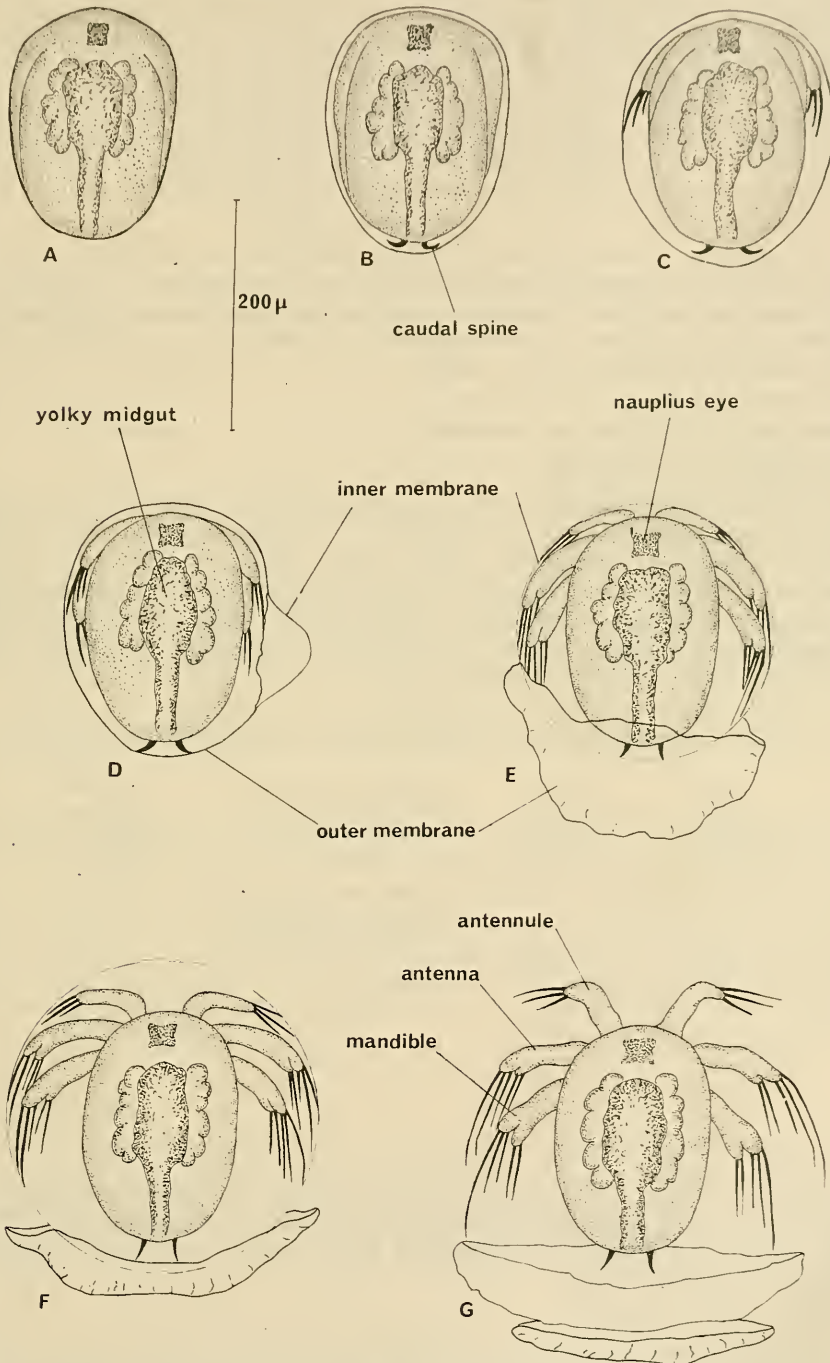


Fig. 2. The hatching sequence of *H. australiensis* (for description, see text).

After this phase of vigorous movement, the nauplius becomes quiescent and a thin film of fluid appears between the nauplius and the egg membranes (Figs 2A, 2B). The increasing volume of the fluid causes the membranes to swell, usually in the equatorial plane. The outer egg membrane ruptures, revealing the transparent inner membrane (Figs 2C, 2D). During this phase of swelling, the naupliar limbs move passively with the expanding inner membrane until they are in the extended swimming position (Fig. 2E). The volume of fluid within the inner membrane continues to increase until the membrane forms a sphere  $270\mu$  in diameter (Fig. 2F). The average time for expansion is 1–2 minutes; and the volume increases by a factor of  $4.3=1$ . The nauplius, which fills the egg membranes before swelling begins, shows no increase in volume during the hatching process.

The nauplius remains quiescent within the inner membrane for a further period of 1–2 minutes after the inner membrane has ceased to expand. The inner membrane is then ruptured as the nauplius swims rapidly forwards (Fig. 2G). The nauplius, freed from its egg membranes, swims immediately to the surface and towards the light.

### *Physical factors influencing hatching*

#### (i) Exposure to bright light:

The marked phototropism of the newly hatched nauplius is preceded by sensitivity to bright light for a number of days before hatching. During those days, muscular twitching of the embryo can be evoked on exposure to flashes of bright light, and the embryo can be stimulated to hatch prematurely by continuous exposure to bright light. The sensitivity to light of embryos from a single egg sac, measured for five days preceding normal hatching, is summarized in Table I.

TABLE I  
*Sensitivity to light of embryos from a single ovisac of Haplostomella australiensis*

Days before normal hatching	Number of embryos tested	Period of exposure to bright light required to initiate hatching (minutes)	Average exposure period (minutes)
5	3	One hatched after 180 minutes. Two failed to hatch in this time	> 180
4	3	120–180	150
3	10	30–90	80
2	15	4–20	15
1	25	1–7	3

As can be seen, the exposure time required to induce hatching falls rapidly as the day of normal hatching approaches. The sensitivity of the twitch response to a flash of bright light also increases during the final days of embryonic development.

#### (ii) Mechanical agitation:

Mechanical agitation also hastens the onset of hatching. In one embryo two days before normal hatching, mechanical agitation with needles under bright light caused hatching to commence after 3 minutes. The average time

of exposure to bright light required to initiate hatching in embryos of this age is 15 minutes, and the minimum time is 4 minutes (Table I). A second embryo, one day before normal hatching, began to hatch while being removed from the ovisac with needles, even before being exposed to bright light.

For 5 embryos spread out in a Petri dish and exposed to bright light, 15 minutes elapsed between the first sign of hatching in the first embryo to hatch and in the last to hatch. For 20 embryos from the same ovisac, kept in contact inside part of the ovisac, the interval was only 6 minutes. In this case, the vigorous prehatching movements of the first embryo to hatch caused mechanical agitation of adjacent embryos and a rapid spread of the hatching response ensued.

### *The synchronization of hatching*

Hatching under bright light stimulation was induced and observed for three intact ovisacs. In each case, the sequence of hatching and its timing for individual embryos within the ovisac was found to be the same as that previously described for individual embryos removed from their ovisacs and studied in isolation. Moreover, the functional significance of the sensitivity of the hatching response to mechanical agitation was revealed by these observations. The vigorous prehatching movements induced by the bright light stimulus began at first in only a few scattered embryos, but spread quickly through adjacent embryos until the whole ovisac vibrated. This mass activity persisted for about 3 minutes, and then almost all of the embryos simultaneously became quiescent as the 1–2 minute phase of expansion of the egg membranes began. The simultaneous increase in volume of the inner membranes, in addition to rupturing the outer membranes, generated a collective pressure sufficient to rupture the ovisac longitudinally. Most of the embryos, still quiescent within their expanding inner membranes, spilled out through the rupture and completed their hatching outside the ovisac. Those remaining within the ovisac hatched and escaped through the longitudinal slit.

The importance of synchronous hatching in promoting escape from the ovisac was confirmed by another experimental observation. Five embryos, one day before normal hatching, were loosely packed and sealed off in part of an ovisac. Simultaneous hatching was then induced by exposure to bright light, but the expansion of the egg membranes was able to be accommodated without rupture of the surrounding ovisac wall. The nauplii thus escaped within the closed ovisac. Although they made vigorous swimming movements, the nauplii were unable to rupture the tough ovisac wall and escape into the surrounding water.

### *Natural hatching*

The hatching of embryos in the laboratory without bright light stimulation was not observed directly, but several batches of nauplii hatched in culture from ovisacs which had been observed to be intact on the previous day. Such hatching was noticed at about 9 a.m., by which time most of the nauplii had entered the quiescent period preceding the moult to a first copepodite (see below). This quiescence follows a 2–3 hour period of activity, indicating that the nauplii had hatched synchronously at about dawn. The associated ruptured ovisacs gave further indication that natural hatching proceeds in the same synchronous manner as experimentally induced hatching.



*Hatching in hypotonic and hypertonic sea water*

## (i) Hypotonic sea water:

The hatching sequence was not altered by immersion of eggs in hypotonic sea water, but the rate of hatching was accelerated (Fig. 3). The duration of both the phase of prehatching activity and the phase of expansion of

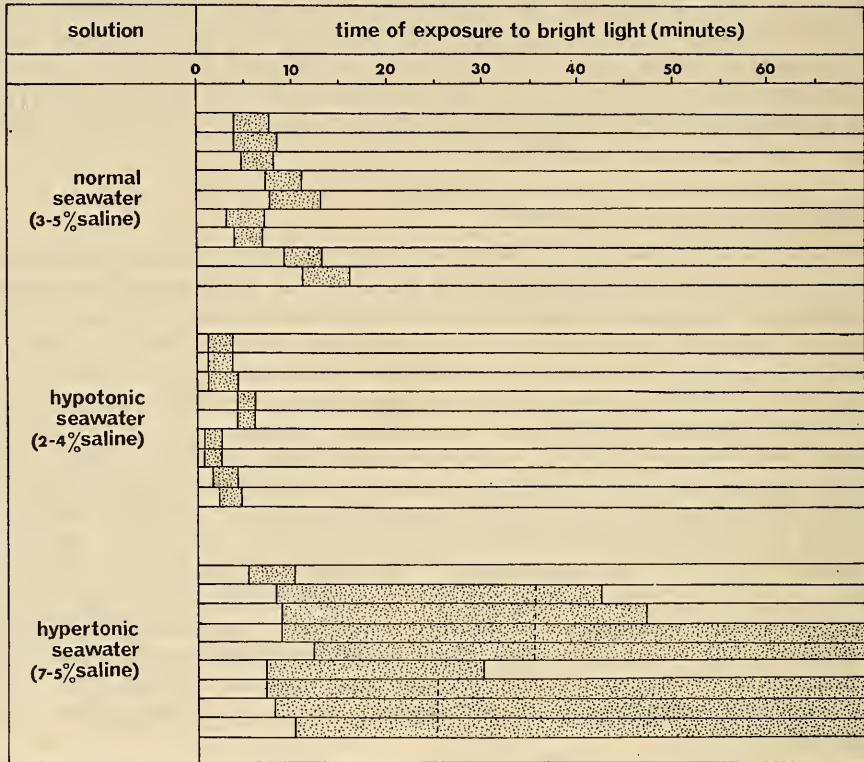


Fig. 3. The timing of light-stimulated hatching of *H. australiensis* in hypotonic and hypertonic sea water, for embryos 2 days before normal hatching. Each shaded block represents the time for one embryo to complete the hatching sequence.

the inner membrane were shortened. When returned to normal sea water after hatching, the nauplii which had been hatched in hypotonic sea water did not differ in behaviour or further development from those hatched in normal sea water.

## (ii) Hypertonic sea water:

Following immersion in hypertonic sea water, both the hatching sequence and the rate of hatching were altered. In response to the stimulus of bright light, the embryos began their prehatching movements in the usual way, but the swelling of the egg membranes was completely inhibited. Only one embryo out of nine attained hatching in the hypertonic solution, in approximately normal time (Fig. 3). Of the remaining 8, three underwent delayed hatching after return to normal sea water, while the remainder failed to hatch. Although prehatching activity was prolonged, no swelling of the egg membranes ensued even after return to normal sea water.

*Larval development*

## (i) The nauplius:

The nauplius, described and illustrated above, remains active at the surface of the water for 2-3 hours after hatching. It then sinks and becomes quiescent, entering the moult to the first copepodite stage.

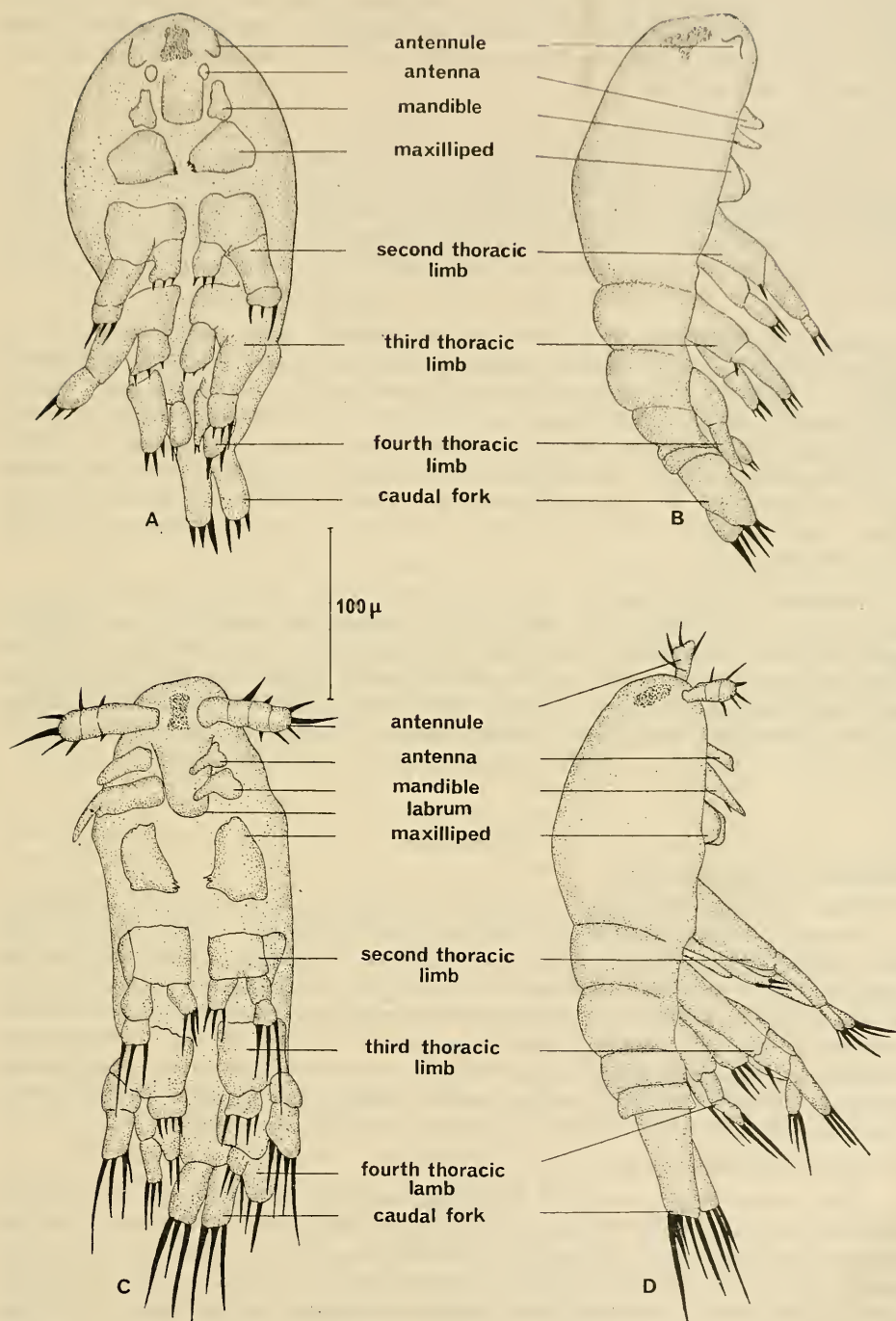


Fig. 4. The first copepodite of *H. australiensis*. A, female, ventral view; B, female, lateral view; C, male, ventral view; D, male, lateral view.



The nauplius swims in the manner typical of newly hatched copepod nauplii (Gauld, 1959), by rapid, synchronous beating of the three pairs of naupliar limbs. Swimming follows a spiral course. At no time does the nauplius display the slower, rhythmic movements typical of the later naupliar stages of planktonic copepods. The main propulsion comes from the action of the long, setose antennae and mandibles, with a lesser contribution from the shorter antennules.

(ii) The first copepodite:

Three to four hours after the onset of quiescence, the nauplius moults, yielding a first copepodite stage (Fig. 4). This larva is 280–300 $\mu$  long. The naupliar appendages are greatly reduced, while the first three pairs of biramous trunk limbs are exposed as functional swimming limbs. The copepodite is not phototropic, and swims at or near the bottom of its container. Probably this stage or the next is the stage which enters the ascidian host.

The first copepodite of *H. australiensis* already shows sexual dimorphism. The male (Figs 4C, 4D) can be diagnostically distinguished from the female (Figs 4A, 4B) by the antennules, which are distinct and segmented in the male, small and inconspicuous in the female, a difference reflecting the specialized later function of the antennules as claspers in male ascidicolid copepods (Lang, 1948). In addition, the remaining naupliar limbs and trunk limbs of the male are generally larger and more setose than those of the female, and a greater space is observed between the maxillipeds and first pair of swimming limbs.

Neither the copepodite stage nor the adult female show traces of maxillules or maxillae. In view of the vermiform character and reduced limbs of the adult female, it is clear that further female larval stages would show the acquisition of two more pairs of trunk limbs, followed by reduction and simplification of the five pairs. The adult male of *H. australiensis* has not been identified, but probably resembles the *Cyclops*-like male of *Ascidicola* (Gotto, 1957) and has a more typical later larval development.

## DISCUSSION

### *Duration of development*

The apparent duration of embryonic development in *H. australiensis*, 6–8 weeks, is comparable with those reported by Wilson (1905, 1907a, 1907b, 1911a, 1911b) for a great number of parasitic copepods. Such periods are long compared with the few days of embryonic development in *Cyclops* and the even shorter 1–2 days in *Calanus* (Marshall and Orr, 1954). The extended embryonic development of parasitic copepods is a reflection of a combination of larger eggs and prolonged direct development to a late larval stage before hatching. *Ascidicola rosea*, with a smaller egg, takes 18 days (Gotto, 1957), and *Gonophysema* 19–20 days (Bresciani and Lützen, 1961).

### *The hatching mechanism*

The sequence of events in hatching, and the operation of the osmotic hatching mechanism in *H. australiensis* are comparable with those described for marine planktonic copepods by Marshall and Orr (1954) and for fresh water copepods by Davis (1959). Each of these shows the phase of vigorous activity just before hatching, the osmotic expansion of the inner membrane and rupture of the outer membrane, and the quiescence of the embryo during expansion of the membranes.

The observation for *H. australiensis* that immersion in hypotonic sea water affects the rate of hatching but not the time of onset of hatching, suggests that the hatching mechanism can be resolved into two components. The first is a light-sensitive component which, when activated, sets in motion the second, an osmotic component. It can be inferred that osmotic stress is set up between the contents of the space between the embryo and its membranes on the one hand, and the surrounding water on the other, as a result of the prehatching activity of the embryo. This activity could result either in secretion of an osmotically active substance or in a change in the structure and permeability of the inner membrane, or both. All that is obvious at the present time is that the activity has a light-sensitive trigger and that the osmotic stress resulting from the activity leads to membrane swelling and escape of the nauplius.

#### *Hatching in natural conditions*

Gotto (1957) observed through the transparent wall of *Corella parallelogramma* (Müller) that *Ascidicola rosea*, which normally inhabits the oesophagus of the host, migrates to the stomach of the host before releasing its ripe egg masses. Here the inner membranes of the eggs swell and burst forth from the clustered outer membranes, but remain intact around the nauplii during passage through the intestine and rectum. Escape of the nauplii from the swollen inner membranes occurs in the exhalant water current of the host.

*Styela etheridgii* has an opaque test, and events intervening between release of the egg sacs by *Haplostomella australiensis* and ensuing natural hatching of the nauplii can only be inferred. In almost 200 ascidian hosts examined, however, no trace was found of detached ovisacs or hatched nauplii of the parasite. This suggests that the ovisacs released by the parasite are carried quickly through the gut and out of the exhalant siphon of the host before hatching takes place. Furthermore, no ovisacs were obtained that hatched normally in less than 9 days. Presumably, ovisacs closer to hatching than 9 days have already been released. The light-sensitivity of hatching is probably significant in preventing hatching before the ovisac has escaped the host since, in contrast to *A. rosea*, the nauplii hatch from their inner membranes as soon as these have swollen and would be exposed to digestive enzymes within the host. Similar light sensitivity in hatching is common among endoparasitic trematodes and cestodes (Smyth, 1961).

Sensitivity to mechanical stimulation probably plays a different role. When the ovisac is released from the female, it is closed off as a tough-walled bag. Experimentation shows that rupture of this bag, essential for the escape of the nauplii, depends on synchronous swelling of the contained egg membranes. The prehatching activity which leads to swelling, although dependent on light stimulation, is accelerated in its onset by mechanical stimulation. Synchrony of the hatching process is assured by this response, since mechanical stimulation spreads rapidly in an ovisac as soon as one embryo makes the prehatching response to light.

During a period of 5 days at 20°C., the embryos become more and more sensitive to both light and mechanical stimulation, until a slight stimulus of either kind will set off synchronous hatching. Presumably this extended gradient of increasing sensitivity permits some margin of delay in escape from the host, while at the same time ensuring eventual hatching even in conditions of relatively low illumination which might be encountered.



Synchronous hatching in parasitic copepods with sac-like ovisacs may be a common phenomenon. Wilson (1911a, 1911b) noted split, empty ovisacs of lernaeopodid and ergasilid copepods after overnight mass hatchings, and also observed that the ovisac ruptured in *Ergasilus* as the egg membranes swelled.

### Larval stages

It is well known that the notodelphyiniform copepods inhabiting ascidians hatch as a phototropic, free-swimming nauplius with reduced limbs and an enlarged, ovoid, yolk-filled body (Canu, 1892; Gray, 1933b; Gurney, 1933; Lang, 1948; Gotto, 1957; Gage, 1966). The Enterocolidae also have a nauplius of this type, although lacking the positive phototropy displayed in the notodelphyiniform species (Canu, 1892; Gray, 1933a; Lang, 1948). The further development of the nauplius has been studied in only a few species (*Ascidicola*, *Notodelphys*, *Enterocola*; Gotto, 1957; Gage, 1966; Canu, 1892) but has been consistently found to pass through four free-swimming naupliar stages and two copepodite stages, of which the second gradually adopts a demersal habit and is probably the infective stage. The free-swimming period of these species is several days.

*Haplostomella australiensis* has a much more abbreviated larval development. The nauplius conforms to the usual type, being similar to that of *Ascidicola rosea*, but is free-swimming for only a few hours before moulting to the demersal first copepodite stage. Furthermore, this stage already manifests sexual dimorphism, which probably indicates that it is the infective stage, as in lernaeopodids (Wilson, 1911a). The only comparable brevity known at the present time for the free-swimming stages of a copepod parasitizing ascidians is that of *Gonophysema gullmarensis*. This aberrant species also has a single, brief naupliar stage and a single copepodite stage infective to new hosts (Bresciani and Lützen, 1961).

### Acknowledgements

We would like to thank Miss S. A. McPhail and Miss S. A. Christmas for technical assistance during the course of this work, Dr. R. V. Gotto, Zoology Department, Queen's University, Belfast, Northern Ireland, for identification of the parasite and Miss I. Bennet, School of Biological Sciences, University of Sydney, Miss E. Pope, Australian Museum, Sydney and Mrs. P. Mather, Department of Zoology, University of Queensland, for identification of the host. The work was supported by research grants from the Australian Research Grants Committee and the University of Sydney.

### References

- BRESCIANI, J., and LÜTZEN, J., 1961.—*Gonophysema gullmarensis* (Copepoda parasitica). An anatomical and biological study of an endoparasite living in the ascidian *Ascidicella aspersa*. II. Biology and development. *Cah. Biol. mar.*, 2: 347-372.
- CANU, E., 1892.—Les copépodes du Boulonnais. *Trav. Stat. zool. Wimereux*, 6: 1-345.
- DAVIS, C. C. 1959.—Osmotic hatching in the eggs of some freshwater copepods. *Biol. Bull. Woods Hole*, 116: 16-22.
- GAGE, J., 1966.—Seasonal cycles of *Notodelphys* and *Ascidicola*, copepod associates with *Ascidicella* (Asciadiacea). *J. Zool.*, 150: 223-233.
- GAULD, D. T., 1959.—Swimming and feeding in crustacean larvae. The nauplius larva. *Proc. zool. Soc. Lond.*, 132: 31-50.
- GOTTO, R. V., 1957.—The biology of a commensal copepod, *Ascidicola rosea* Thorell, in the ascidian *Corella parallelogramma* (Müller). *J. mar. biol. Ass. U.K.*, 36: 281-290.
- , 1969.—*Haplostomella australiensis*, n. sp., an ascidicolous copepod from New South Wales. *Crustaceana*, in press.



- GRAY, P., 1933a.—The nauplii of *Notodelphys agilis* Thorell and *Doropygus porcicauda* Brady. *J. mar. biol. Ass. U.K.*, 18: 519-522.
- , 1933b.—*Mycophilus rosovula* n. sp., a notodelphoid copepod parasitic within *B. (Botrylloides) leachii* Sav., with a description of the nauplius and notes on its habits. *J. mar. biol. Ass. U.K.*, 18: 523-527.
- GURNEY, R., 1933.—Notes on some Copepoda from Plymouth. *J. mar. biol. Ass. U.K.*, 19: 229-304.
- LANG, K., 1948.—Copepoda "Notodelphyoida" from the Swedish west coast, with an outline of the systematics of the copepods. *Ark. Zool.*, 40: 1-36.
- MARSHALL, S. M., and ORR, A. P., 1954.—Hatching in *Calanus finmarchicus* and some other copepods. *J. mar. biol. Ass. U.K.*, 33: 393-401.
- SMYTH, J. D., 1962.—"An introduction to animal parasitology." London, English Univ. Press.
- WILSON, C. B., 1905.—North American parasitic copepods belonging to the family Caligidae. Part I. The Caliginae. *Proc. U.S. nat. Mus.*, 28: 479-672.
- , 1907a.—North American parasitic copepods belonging to the family Caligidae. Part 2. The Trebinae and Euryphorinae. *Proc. U.S. nat. Mus.*, 31: 669-720.
- , 1907b.—A revision of the Pandarinae and Cecropinae. *Proc. U.S. nat. Mus.*, 31: 323-490.
- , 1911a.—North American copepods belonging to the family Lernaeopodidae. *Proc. U.S. nat. Mus.*, 39: 186-226.
- , 1911b.—North American copepods belonging to the family Ergasilidae. *Proc. U.S. nat. Mus.*, 39: 263-400.

# HATCHING AND LARVAL DEVELOPMENT OF *DISSONUS NUDIVENTRIS* KABATA (COPEPODA, FAM. DISSONIDAE),

## A GILL PARASITE OF THE PORT JACKSON SHARK

D. T. ANDERSON and G. T. ROSSITER

*School of Biological Sciences, University of Sydney*

[Read 27th November, 1968]

### *Synopsis*

*D. nudiventris* lays typical, uniseriate, caligid egg strings, but hatches as a nauplius which remains attached to the egg-string by paired caudal threads and has reduced naupliar limbs. The nauplius moults, yielding a free-swimming, demersal copepodite which is probably the infective stage of the life cycle. The peculiarities of hatching and development in *D. nudiventris* support the removal of the genus from the Caligidae to the monogeneric family Dissonidae, proposed on morphological grounds by Yamaguti.

### INTRODUCTION

While the number of specific descriptions of copepods parasitizing fishes in Australian waters is gradually increasing (Heegaard, 1962; Kabata, 1965, 1966), nothing has been recorded of their larval stages. The genus *Dissonus*, comprising a small number of caligoid species associated with elasmobranch fishes, is a particularly interesting one. Created by Wilson (1906), this genus remained within the Caligidae until 1963, when it was removed by Yamaguti (1963) to a separate family Dissonidae on the basis of a number of small but significant morphological differences. *Dissonus nudiventris* was first collected by the British, Australian and New Zealand Antarctic Expedition of 1929–1931, from *Heterodontus phillipi* Blainville at Hobart, Tasmania, but the material was described and named as a new species only by Kabata (1965). Although Kabata followed Yamaguti in assigning the species to the family Dissonidae, the first words of his specific description read “A typical caligid copepod———”, emphasizing the fact that the adults of *D. nudiventris* are similar in general form to the well known caligids.

Wilson (1905, 1907*a*, 1907*b*), Gurney (1934) and Heegaard (1947) have stressed the uniformity in structure and habits of caligid larvae. The following description of the hatching and early larval development of *D. nudiventris* shows that in Yamaguti's newly proposed family, larval development differs markedly in the early stages from that of the Caligidae.

### MATERIALS AND METHODS

Male and female adults of *Dissonus nudiventris* were collected from the gill filaments of the Port Jackson shark, *Heterodontus portjacksoni* (Meyer). The fishes came from the marine aquarium at Manly, N.S.W. Of four individuals examined, one yielded 23 females and 6 male parasites, a second 65 females only and a third 1 female only, while the fourth was uninfested.

In all, fourteen egg strings of *D. nudiventris* containing advanced embryos were cultured. The egg strings were found to be highly susceptible to bacterial and protozoan attack in the conditions of the culture. Using movements of the gut in the embryos as a convenient index of viability, it

was found that the embryos remained alive for no more than five days. Hatching was observed only if it took place during this period. Numerous nauplii were hatched, but very few survived the first moult to the first copepodite stage, and no larvae were carried beyond this stage.

In spite of these difficulties, which are well known for parasitic copepods (Wilson, 1905, 1907*a*, 1907*b*; Heegaard, 1947), the results obtained revealed a unique pattern of hatching and larval development in *Dissonus nudiventris*, quite unlike that of any caligid.

## RESULTS

### The egg string

*D. nudiventris* produces paired egg strings of typical caligid form. Each egg string is cylindrical, 4–6 mm. long, 0.35 mm. in diameter, and contains a single row of flattened, disc-shaped eggs, closely packed together with their flattened surfaces almost touching. Each egg lies in a separate cell of the egg string, with diameters of  $350 \times 320\mu$  and a thickness of  $70\mu$ . The wall of the cell is relatively rigid and tough. As the embryos develop, it can be seen that the flattening of the egg is a dorso-ventral one. The dorsal surface

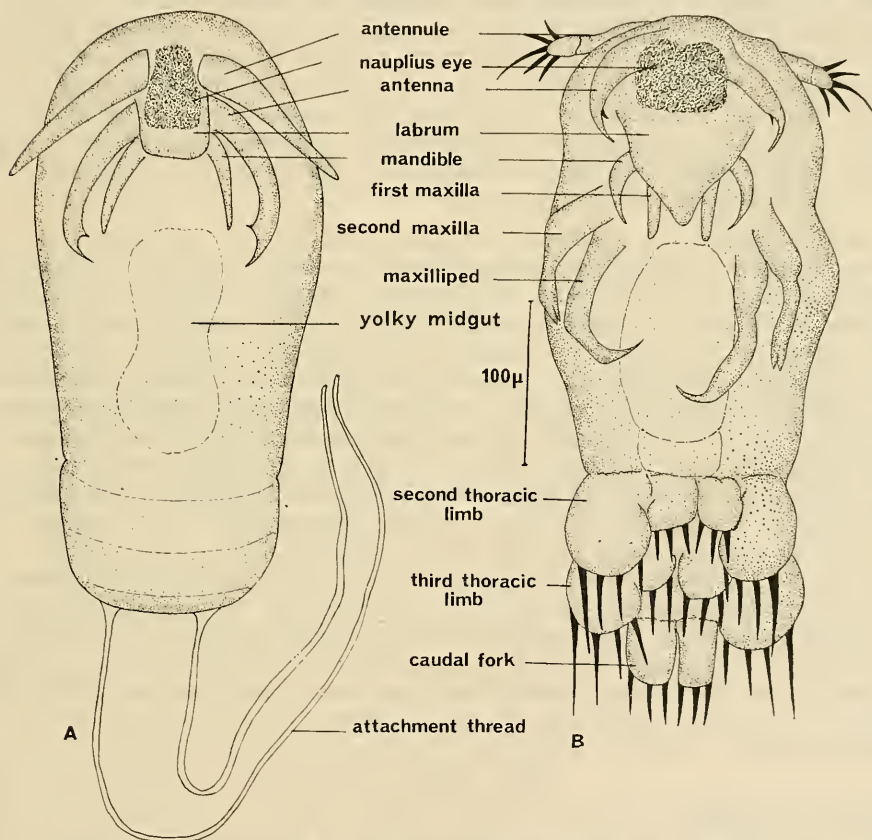


Fig. 1. *Dissonus nudiventris*. A. The newly hatched nauplius, ventral view. B. The first copepodite, ventral view.

of each embryo faces the ventral surface of the next embryo of the string. Furthermore, the anterior ends of the embryos, marked in later stages by the nauplius eyes, are also aligned. The embryos all face in one direction along the length of the string.



### *The newly hatched larva*

The newly hatched nauplius which emerges from a cell of the egg string in *D. nudiventris* is unique among Crustacea. Although diagnostically a nauplius, with 3 pairs of naupliar limbs and an externally unsegmented post-naupliar region (Fig. 1A), it is non-feeding and non-swimming. The nauplius remains permanently attached to the wall of the egg string by a pair of fine, fibrous threads arising from the posterior end of the larva. The post-naupliar region is elongated, and the naupliar limbs are short, devoid of swimming setae or feeding spines, ventrally disposed and generally similar to the form they take in the next larval stage.

### *Hatching*

Hatching of each individual nauplius from its cell in the egg string is a relatively prolonged process. A variable period of activity, which may continue sporadically for many minutes, precedes hatching. The first sign of hatching is a slight swelling of the membrane around the embryo, accompanied by formation of a film of fluid between the embryo and the membrane (Figs 2A, 2B). The embryo rounds up slightly from its previous flattened form. Further swelling of the membrane, resisted by the tough wall of the cell, gradually carries the nauplius out through an aperture formed in the wall of the cell, immediately in front of the nauplius eye (Fig. 2C). The formation of this aperture is imperceptible. Vigorous movements of the embryo occur during this phase of hatching. The increase in volume of the membrane is approximately fourfold.

As the nauplius is gradually extruded from its cell, the caudal threads become apparent (Fig. 2C) and gradually extend (Fig. 2D). When the swollen membrane breaks and the nauplius finally emerges, the threads continue to restrain it. At this stage, they measure about twice the length of the nauplius, which itself is about  $370\mu$  long. The nauplius continues to twitch its limbs and body after hatching, but makes no swimming movements and shows no photosensitivity. The duration of hatching of individual nauplii emerging from three different egg strings ranged from a few minutes to three hours. Hatching within any one egg string was asynchronous and was not promoted by exposure to light. It was, however, sensitive to the osmotic concentration of the surrounding medium. Immersion of part of an egg string, from which some nauplii had already hatched, in hypotonic sea water (2.4% saline), caused 10 more nauplii to hatch within 5 minutes, a degree of synchrony never observed in normal hatching. Conversely, immersion of another part of the same egg string in hypertonic sea water (7.5% saline) inhibited all further hatching. It can be inferred that, as in other copepods (Marshall and Orr, 1954; Davis, 1959; Anderson and Rossiter, 1968), the swelling of the egg membrane during hatching in *D. nudiventris* has an osmotic basis.

### *Larval development*

#### (i) The nauplius:

The nauplius (Fig. 1A) remains unchanged in external appearance until it moults, after 2–5 hours, into a first copepodite. The nauplius remains attached to the egg string by the caudal threads, and shows only sporadic twitching. The large, curved, spatulate caudal spines of the typical caligid free-swimming nauplius, referred to by Wilson (1905) as balancing organs, are conspicuously absent.

## (ii) The first copepodite:

The first copepodite (Fig. 2B) emerges through a longitudinal split in the anterior dorsal midline of the naupliar cuticle, leaving the exuvia intact and still attached to the egg string by the paired caudal threads. The first copepodite retains the same size and general shape as the nauplius, but shows three new features:

(a) further modification of the naupliar limbs. The antennules become segmented and setose. The antennae become more hook-like, approximating to their adult form. The stylet-like mandibles come into closer association with the labrum, which itself becomes larger and pointed;

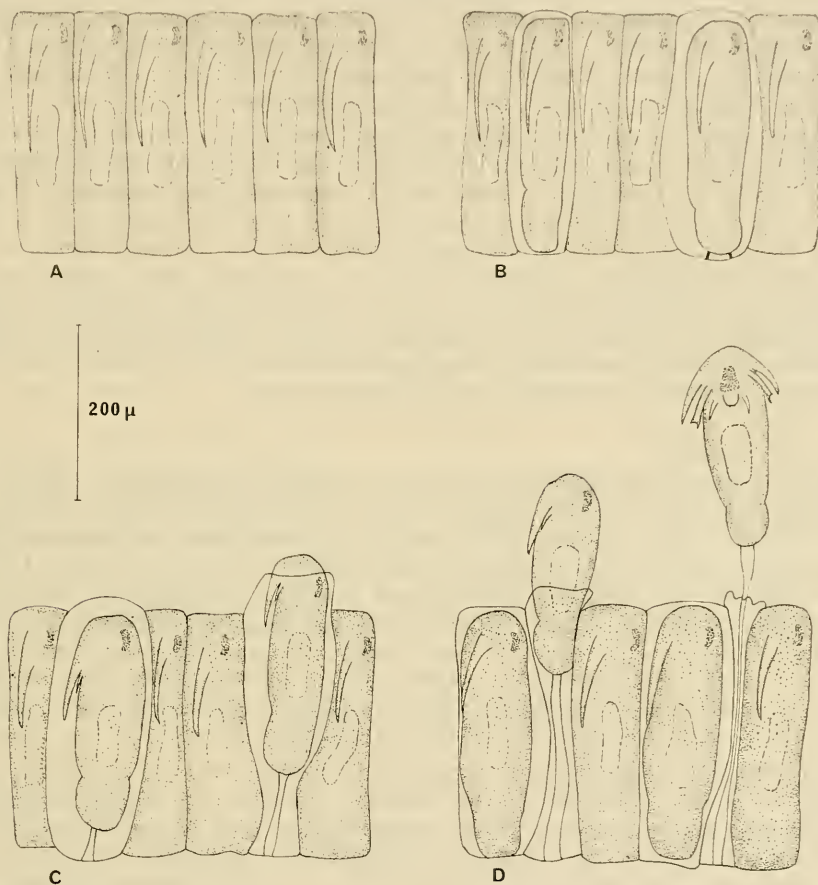


Fig. 2. The hatching sequence of *Dissonus nudiventris* (for description, see text).

(b) emergence of the maxillules, as stylets associated with the labrum and of elongated maxillae with chelate tips;

(c) emergence of three trunk segments, with a pair of long clawed maxillipeds anteriorly on the first and two pairs of functional, biramous swimming limbs posteriorly on the second and third segments.

The first copepodite is lecithotrophic, free-swimming and demersal, swimming about near the bottom of the container. There is no sign of a frontal filament, the thread by which typical caligid copepods (the chalimus stage) attach themselves to their hosts before completion of the larval stages (Wilson, 1907; Gurney, 1934; Heegaard, 1947; Baer, 1952).

No sexual dimorphism is observed in the first copepodite of *D. nudiventris* but, as noted by Kabata (1965), sexual dimorphism is not marked in the adults of this species. Apart from differences in proportion in the genital segments, the adult female differs from the male only in the absence of the vestiges of a sixth pair of thoracic limbs.

## DISCUSSION

### *The egg strings and hatching*

The egg strings of *D. nudiventris* are similar to those of the many caligid species described by Wilson (1905) and others. The sensitivity of the embryos and larvae in conditions of artificial culture also reiterates the findings of Wilson and of Heegaard (1947) for the eggs of many copepods parasitizing fish. Generally, these parasites experience very efficient ventilation, the absence of which may be a critical factor in culture.

Associated with this difficulty, the long duration of hatching from the egg string in *D. nudiventris* may not be a true indication of hatching in natural conditions. Wilson (1905, 1907) observed that hatching from a typical caligid egg string takes place more or less simultaneously, and in view of the synchrony of development of the eggs in the egg string of *D. nudiventris*, synchronous hatching is to be expected. Since each embryo emerges independently from its own cell in the egg string, however, the role of synchronous hatching in rupturing the ovisac in the ascidicolid copepod *Haplostomella australiensis* and other species with sac-like ovisacs (Anderson and Rossiter, 1968) is irrelevant in *D. nudiventris*.

### *Larval development*

While passage through most or all of naupliar development within the egg membranes is a feature of lernaeid and lernaeopodid copepods parasitizing fishes (Wilson, 1911a; Baer, 1952) and of *Gonophysema gullmarensis* and *Haplostomella australiensis* parasitizing ascidians (Bresciani and Lützen, 1961; Anderson and Rossiter, 1968) there has been no previous record of a non-swimming nauplius which hatches and yet remains attached by paired threads to the egg string, as in *D. nudiventris*. This nauplius is a remarkable exception to the rule noted by C. B. Wilson (1911a) that "all copepod nauplii, as well those of parasitic forms as those of free swimmers, seek the surface of the water and there swim about freely".

The brief attached naupliar stage of *D. nudiventris* can be interpreted as an exotic means of shortening the duration of free larval stages, alternative to the prolonged direct development within the egg seen in lernaeopodids and many other Crustacea. It contrasts strongly with the typical succession of two phototropic, free-swimming naupliar stages and two demersal copepodite stages seen in caligid development (Heegaard, 1947). The caligid larvae provide a prolonged dispersal and host-seeking phase in the life cycle. The elimination of the free-swimming naupliar stages in *D. nudiventris*, with retention of the demersal first copepodite, greatly restricts dispersal, apparently in favour of protected, more direct development. This loss may not be important, however, since both male and female adults of *D. nudiventris* can leave the host and swim freely, at least for short periods.

Associated with the loss of naupliar swimming, the naupliar limbs develop directly towards their later adult form, and the first copepodite already has mouthparts approximating in structure to those of the adult. Since the larva is also lecithotrophic, slow-swimming and demersal, it seems likely to be the immediate host-seeking and attachment stage. It follows that the



peculiar attached nauplius is in all probability an adaptation to completion of the life cycle of *D. nudiventris* on a single host shark, with the emergent first copepodite stage attaching directly to the gills on to which it emerges. This might well be advantageous in view of the solitary habits and scattered distribution of the Port Jackson shark, leaving host transfer to the adult during moments of host contact, e.g. in mating. Infestation by one ovigerous female would then be sufficient to ensure rapid parasitization of a previously unparasitized host. It is notable that of the sharks examined, one was totally uninfested.

### Acknowledgements

We would like to thank Miss S. A. McPhail and Miss S. A. Christmas for technical assistance during the course of this work, Professor G. H. Satchell, School of Biological Sciences, University of Sydney, for making available the Port Jackson Sharks, and Mr. G. C. Hewitt, Zoology Department, Victoria University of Wellington, New Zealand, for the identification of the parasite. The work was supported by research grants from the Australian Research Grants Committee and the University of Sydney.

### References

- ANDERSON, D. T., and ROSSITER, G. T., 1968.—Hatching and larval development of *Haplostomella australiensis* Gotto (Copepoda, Fam. Ascidicolidae), a parasite of the ascidian *Styela etheridgii* Herdman. *Proc. Linn. Soc. N.S.W.*, 93: 464–475.
- BAER, J. G., 1952.—“Ecology of animal parasites.” Urbana. Univ. of Illinois Press.
- BRESCIANI, J., and LÜTZEN, J., 1961.—*Gonophysema gullmarensis* (Copepoda parasitica). An anatomical and biological study of an endoparasite living in the ascidian *Asciidiella aspersa*. II. Biology and development. *Cah. Biol. mar.*, 2: 347–372.
- DAVIS, C. C., 1959.—Osmotic hatching in the eggs of some freshwater copepods. *Biol. bull. Woods Hole*, 116: 15–29.
- GURNEY, R., 1934.—The development of certain parasitic Copepoda of the families Caligidae and Clavellidae. *Proc. zool. Soc. Lond.*, 1934, 177–217.
- HEEGAARD, P., 1947.—Contributions to the phylogeny of the arthropods. Copepoda. *Spolia Zool. Mus. Hauniensis*, 8: 1–236.
- , 1962.—Parasitic Copepoda from Australian fishes. *Rec. Aust. Mus.*, 25: 149–234.
- KABATA, Z., 1965.—Parasitic Copepoda of fishes. *B.A.N.Z. Antarctic Research Expdn., 1929–31. Reports, Ser. B (Zool. and Bot.)*, 8 (6): 1–16.
- , 1966.—Copepoda parasitic on Australian fishes. VI. Some caligoid species. *Ann. Mag. Nat. Hist., Ser. 13*, 9: 563–70.
- MARSHALL, S. M., and ORR, A. P., 1954.—Hatching in *Calanus finmarchicus* and some other copepods. *J. mar. biol. Ass. U.K.*, 33: 393–401.
- WILSON, C. B., 1905.—North American parasitic copepods belonging to the family Caligidae. Part I. The Caliginae. *Proc. U.S. nat. Mus.*, 28: 479–672.
- , 1906.—Report on some parasitic Copepoda collected by Prof. Herdman at Ceylon, 1902. *Rept. Govt. Ceylon Pearl Oyster Fish. Gulf Mannar (Herdman)* Pt. 5: 189–210.
- , 1907a.—North American parasitic copepods belonging to the family Caligidae. Part 2. The Trebinae and Euryphorinae. *Proc. U.S. nat. Mus.*, 31: 669–720.
- , 1907b.—A revision of the Pandarinae and Cecropinae. *Proc. U.S. nat. Mus.*, 33: 323–490.
- , 1911a.—North American parasitic copepods belonging to the family Lernaeopodidae. *Proc. U.S. nat. Mus.*, 39: 186–226.
- , 1911b.—North American parasitic copepods belonging to the family Ergasilidae. *Proc. U.S. nat. Mus.*, 39: 263–400.
- YAMAGUTI, S., 1963.—“Parasitic Copepoda and Branchiura of fishes.” New York. Interscience.